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L5: Entry 24 of 28

File: USPT

Jul 27, 1993

DOCUMENT-IDENTIFIER: US 5230899 A

TITLE: Methods and compositions for making liposomes

Brief Summary Text (16):

In practical terms, liposomes formed using this invention are formulated as a "pre-liposome gel" referred to herein as a "gel" where a phospholipid and an aliphatic or aromatic-based acid or amine mixture capable of forming liposomes is mixed with an appropriate, concentrated aqueous solution of the hydrating compound. This gel, upon dispersion in an aqueous solution, efficiently and spontaneously forms liposomes without solvent evaporation, input of ultrasonic irradiation or any of the other means developed to insure proper formation of lipid vesicles, liposomes.

Brief Summary Text (18):

Additionally, the pre-liposome gel can be dehydrated and stored for a substantial period of time and still be capable of spontaneously forming liposomes upon rehydration.

Brief Summary Text (19):

The pre-liposome gel is extraordinarily stable, stable enough to be autoclaved for sterilization. Furthermore, water-soluble or water-insoluble substances to be encapsulated can be added to the gel and will then be incorporated into the liposomes upon dispersion of the gel. This capability has the effect of greatly enhancing the encapsulation efficiency.

Brief Summary Text (43):

Examples of liposome-forming materials include saponifiable and non-saponifiable lipids, e.g., the acyl glycerols, the phosphoglycides, sphingolipids, the glycolipids, etc. fatty acids include saturated or unsaturated alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) carboxylic acids, mono-alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.27</sub>) esters of C.<sub>sub.4</sub> .about.C.<sub>sub.10</sub> dicarboxylic acids (e.g., cholesterol hemi-succinic acid and fatty acid derivatives of amino acids in which any N-acyl carboxylic acids also are included (e.g., N-oleoyl threonine, N-linoleoyl serine, etc.). Mono- or di-alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) sulfonate esters and mono- or di-alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) phosphate esters can be substituted for the fatty acids. Furthermore, mono- or di-acyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) glycerol derivatives of phosphoric acids and mono- or di-acyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) glycerol derivatives of sulfuric acids can be used in place of the fatty acids.

Brief Summary Text (44):

Additionally, the fatty acids also can be replaced by amines (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> NH.<sub>sub.2</sub>), C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> fatty acid derivatives of amines (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> CONH.about.NH.<sub>sub.2</sub>), C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> fatty alcohol derivatives of amino acids (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> OOC.about.NH.<sub>sub.2</sub>), and C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> fatty acid esters of amines (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> COO.about.NH.<sub>sub.2</sub>).

Brief Summary Text (46):

Although the primary components of these liposomes will be lipids, phospholipids, other fatty acids, there may also be added various other components to modify the liposomes' permeability. There may be added, for example, non-ionic lipid

components such as polyoxy alcohol compounds, polyglycerol compounds or esters of polyols; the esters of polyols and synthetic lipolipids, such as cerebrosides. Other materials, such as long chain alcohols and diols, sterols, long chain amines and their quaternary ammonium derivatives; polyoxyethylenated fatty amines, esters of long chain amino alcohols and their salts and quaternary ammonium derivatives; phosphoric esters of fatty alcohols, polypeptides and proteins.

Brief Summary Text (48):

It also has been discovered that if the lipid component itself or the substances (e.g., medicaments, biologically active compounds, cosmetics, etc.) to be encapsulated possess the aforementioned properties, the lipid composition may not require the inclusion of the fatty acids (or the amines) or the hydrating agents to form the "pre-liposome gel". For example, the mixture of dipalmitoylphosphatidylcholine (DPPC) and distearoyl phosphatidylethanolamine forms the "pre-liposome gel" or liposomes with aqueous glutamic acid solution and the mixture of DPPC and oleic acid with aqueous epinephrine solution forms the "pre-liposome gel" and liposomes.

Brief Summary Text (74):

Mixtures of liposome-forming materials, a long chain aliphatic or aromatic-based acid or amine, and one or more hydrating agents with up to 300 moles of water relative to the total solids gives a gel which forms liposomes directly therefrom upon addition of an aqueous solution. This gel can be labeled a pre-liposome gel because i.) of its structural characteristics which are essentially those of liposomes and, ii.) the gel's facility for being converted into liposomes upon dilution with an aqueous solution. Aqueous solution in excess of about 300 moles cause the beginning of liposome formation.

Brief Summary Text (79):

The pre-liposome gel, with or without the material to be encapsulated, also can be dehydrated (e.g. lyophilized) and the powder rehydrated to form liposomes spontaneously, even after a long period of storage. This capability makes the invention particularly useful for administering water-sensitive medicaments where long term pre-use storage is needed.

Detailed Description Text (12):

ii). Manufacture of Liposomes: The gel prepared in the preceding Paragraph was taken from cold storage and returned to room temperature. It was then mixed with 2 liters of phosphate buffered saline, pH 7.4. A white opaque liposome solution was formed.

Detailed Description Text (17):

Pre-Liposome Gel

Detailed Description Text (53):

To 120 mg of dipalmitoylphosphatidylcholine was added 40 mg of oleic acid to form a homogeneous paste. Forty mg of pilocarpine free base was added to 10 ml of distilled deionized water. This solution was added to the paste and heated to 45.degree. C. to form a pre-liposome gel. The resulting gel was diluted with 20 ml of phosphate buffered saline to form liposomes.

Detailed Description Text (72):

Sterile liposomes may be prepared from the heat sterilized pre-liposome gel. Alternatively, the liposome gel or the liposomes may be sterile filtered through an appropriate sterilizing filter.

Detailed Description Text (84):

A 10.0 gm aliquot of this pre-liposome gel was transferred to a 10 ml vial and lyophilized. The resulting powder formed liposomes when diluted with 5 ml of phosphate buffered saline.

Current US Original Classification (1) :  
424/450

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L5: Entry 14 of 28

File: USPT

Oct 13, 1998

US-PAT-NO: 5820848

DOCUMENT-IDENTIFIER: US 5820848 A

**TITLE:** Methods of preparing interdigititation-fusion liposomes and gels which encapsulate a bioactive agent

**DATE-ISSUED:** October 13, 1998

**INVENTOR-INFORMATION:**

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boni; Lawrence T.	Monmouth Junction	NJ		
Janoff; Andrew S.	Yardley	PA		
Minchey; Sharma R.	Monmouth Junction	NJ		
Perkins; Walter R.	Monmouth Junction	NJ		
Swenson; Christine E.	Princeton Junction	NJ		
Ahl; Patrick L.	Princeton	NJ		
Davis; Thomas S.	Valhalla	NY		

**US-CL-CURRENT:** 424/9.4; 264/4.1, 424/1.21, 424/450, 424/9.321, 436/829, 516/102

**CLAIMS:**

What is claimed is:

1. A method of producing a pharmaceutical composition comprising an interdigititation-fusion liposome and a bioactive agent which comprises: (a) preparing sized liposomes having an average diameter of less than about 0.05 microns and comprising a symmetrical saturated phospholipid; (b) combining the sized liposomes with an amount of an inducer effective to induce fusion of the sized liposomes and interdigititation of the saturated phospholipid so as to form an interdigititation-fusion gel from the sized liposomes; (c) adding a bioactive agent to the gel; (d) incubating the interdigititation-fusion gel at a temperature above the transition temperature of the saturated phospholipid in the gel for a period of time effective to form an interdigititation-fusion liposome from the gel; and, (e) combining the interdigititation-fusion liposome and a pharmaceutically acceptable carrier, wherein the inducer is selected from the group consisting of short-chain alcohols, polyols, chaotropic salts and aqueous buffers and wherein the interdigititation-fusion liposome comprises the bioactive agent at an agent-to-lipid ratio (w/w) of at least about 2:1.

2. The method of claim 1 further comprising adding an additional lipid to the combination of liposomes and inducer prior to incubation.

3. The method of claim 2, wherein the additional lipid is a noninterdigitating lipid.

4. The method of claim 1, wherein the inducer is a short chain alcohol

selected from the group consisting of methanol, ethanol, propanol and n-butanol.

5. The method of claim 4, wherein the inducer is ethanol.

6. The method of claim 5, wherein the effective amount of ethanol is an amount equal to about 5% of the weight of the lipid in the sized liposomes to about 20% of the weight of the lipid..

7. The method of claim 6, wherein the effective amount of ethanol is an amount equal to about 7% of the weight of the lipid in the sized liposomes.

8. A method of preparing a pharmaceutical composition comprising an interdigitation-fusion liposome and a bioactive agent which comprises: (a) preparing sized liposomes having an average diameter of less than about 0.05 microns and comprising a symmetrical saturated phospholipid; (b) subjecting the sized liposomes to a hydrostatic pressure of at least about 10,000 psi for a period of time sufficient to fuse the sized liposomes and interdigitate the saturated phospholipid, so as to form an interdigitation-fusion gel from the sized liposomes; (c) adding a bioactive agent to the gel; (c) incubating the interdigitation-fusion gel at a temperature above the transition temperature of the saturated phospholipid in the gel for a period of time effective to form an and, interdigitation-fusion liposome from the gel; (e) combining the liposome and a pharmaceutically acceptable carrier, wherein the interdigitation-fusion liposome comprises the bioactive agent.

9. The method of claim 8, wherein the amount of the hydrostatic pressure is at least about 20,000 psi.

10. The method of claim 9, wherein the amount of the hydrostatic pressure is at least about 40,000 psi.

11. The method of claim 8, wherein the amount of hydrostatic pressure is effective to sterilize the gel.

12. The method of claim 8, wherein the hydrostatic pressure is applied for a period of time of from about 1 minute to about 1 hour.

13. A method of producing a pharmaceutical composition comprising an interdigitation-fusion liposome and a bioactive agent which comprises: (a) preparing sized liposomes having an average diameter of less than about 0.05 microns and comprising a symmetrical saturated phospholipid; (b) incubating the sized liposomes for a period of time of from about 1 minute to about 1 hour, so as to form an interdigitation-fusion gel from the sized liposomes; (c) adding a bioactive agent to the gel; (d) incubating the interdigitation-fusion gel at a temperature above the transition temperature of the self-inducing lipid in the gel for a period of time effective to produce an interdigitation-fusion liposome from the gel; and, (e) combining the liposome and a pharmaceutically acceptable carrier, wherein the interdigitation-fusion liposome comprises the bioactive agent at an agent-to-lipid ratio (w/w) of at least about 2:1.

14. The method of claim 13, wherein the self-inducing lipid is di-O-hexadecyl phosphatidylcholine.

15. The method of claim 13, wherein the sized liposomes are incubated for from about 1 minute to about 1 hour.

16. The composition of claim 1, wherein the sized liposomes have an average diameter of about 0.025 microns, the saturated phospholipid is dipalmitoyl phosphatidylcholine, the inducer is ethanol and the amount of ethanol in the composition is from about 5% to about 20% by weight of the lipid.

17. The composition of claim 16, wherein amount of ethanol in the composition is about 17% by weight of the lipid.

18. The method of claim 1, wherein the symmetrical saturated phospholipid is selected from the group consisting of dimyristoyl phosphatidylcholine, distearoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylserine, dipalmitoyl phosphatidylserine, distearoyl phosphatidylserine, dimyristoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, distearoyl phosphatidylethanolamine, dimyristoyl phosphatidic acid, distearoyl phosphatidic acid, dipalmitoyl phosphatidic acid, dimyristoyl phosphatidylinositol, distearoyl phosphatidylinositol, dipalmitoyl phosphatidylinositol, hydrogenated soy phosphatidylcholine, dipalmitoyl phosphatidylglycerol, di-O-hexadecyl phosphatidylcholine, distearoyl phosphatidylglycerol and dimyristoyl phosphatidylglycerol.

19. The method of claim 8, wherein the symmetrical saturated phospholipid is selected from the group consisting of dimyristoyl phosphatidylcholine, distearoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylserine, dipalmitoyl phosphatidylserine, distearoyl phosphatidylserine, dimyristoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, distearoyl phosphatidylethanolamine, dimyristoyl phosphatidic acid, distearoyl phosphatidic acid, dipalmitoyl phosphatidic acid, dimyristoyl phosphatidylinositol, distearoyl phosphatidylinositol, dipalmitoyl phosphatidylinositol, hydrogenated soy phosphatidylcholine, dipalmitoyl phosphatidylglycerol, di-O-hexadecyl phosphatidylcholine, distearoyl phosphatidylglycerol and dimyristoyl phosphatidylglycerol.

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L4: Entry 43 of 81

File: USPT

Jul 18, 2000

US-PAT-NO: 6090955

DOCUMENT-IDENTIFIER: US 6090955 A

TITLE: Liposome-encapsulated taxol, its preparation and its use

DATE-ISSUED: July 18, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Reszka; Regine	Schwanebeck			DE
Brandl; Martin	Freiburg			DE
Fichtner; Iduna	Berlin			DE
Warnke; Gernot	Freiburg			DE

US-CL-CURRENT: 549/510

## CLAIMS:

We claim:

1. A liposome-encapsulated taxol.

2. The liposome-encapsulated taxol of claim 1, comprising as encapsulating agent

a) a natural, semi-synthetic or fully synthetic amphiphilic material,  
b) one or more of a charged lipid component, a saturated lipid component and an ether lipid component

c) a polymer and

d) a carrier liquid.

3. The liposome-encapsulated taxol of claim 1, wherein it contains a natural, semi-synthetic or fully synthetic amphiphilic compound of formula ##STR1## wherein R.<sub>sub.1</sub> and R.<sub>sub.2</sub> represent C.<sub>sub.10</sub> to C.<sub>sub.20</sub> alkanoyl, alkenoyl, alkyl or alkenyl.

4. The liposome-encapsulated taxol of claim 1, wherein, as charged lipid component, it contains one chosen from the group consisting of the anion of dicetyl phosphate, palmitic acid, stearic acid, the anion of a phospholipid, phosphatide acid, and the anion of a sphingolipid.

5. The liposome-encapsulated taxol of claim 1, wherein it contains, as charged lipid components, a chemically modified phosphatidyl ethanolamine, over which

proteins can be coupled.

6. The liposome-encapsulated taxol of claim 1, wherein it contains phosphatidyl choline as neutral lipid components.
7. The liposome-encapsulated taxol of claim 1, wherein it contains phosphatidyl serine as charged lipid components.
8. The liposome-encapsulated taxol of claim 1, wherein it contains phosphatidyl glycerol as charged lipid components.
9. The liposome-encapsulated taxol of claim 1, wherein it contains dipalmitoyl phosphatidyl choline as saturated lipid components.
10. The liposome-encapsulated taxol of claim 1, wherein it contains dimyristoyl phosphatidyl choline as saturated lipid components.
11. The liposome-encapsulated taxol of claim 1, wherein it contains ether lipids as charged lipid components.
12. The liposome-encapsulated taxol of claim 1, wherein it contains polyethylene glycol in or at the membrane of the vesicle.
13. A pharmaceutical preparation containing an effective amount of encapsulated taxol of claims 1 and pharmaceutically conventional carriers and additives.
14. A method for the preparation of liposome-encapsulated taxol of claim 1, wherein a mixture of membrane-forming amphiphiles, in which taxol was dissolved, and an aqueous phase, followed by removal of the solvent by evaporation and dispersal in water, is subjected one or more times but not more than fifty times to a high-pressure homogenization at pressures of 50 to 1,600 bar.
15. A method for producing liposome-encapsulated taxol by aerosol formation, wherein a previously produced liposome mixture in solid or liquid form is combined with taxol and subsequently treated in special aerosol forming equipment.
16. A method for producing liposome-encapsulated taxol, wherein taxol and the encapsulating agent are present in dissolved form in a pressure-liquefied blowing gas and, after evaporation of the blowing gas, are converted to encapsulated taxol by spontaneous vesicle formation on the epithelium of the lungs.

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L4: Entry 76 of 81

File: USPT

Jul 27, 1993

DOCUMENT-IDENTIFIER: US 5230899 A

TITLE: Methods and compositions for making liposomes

Brief Summary Text (16):

In practical terms, liposomes formed using this invention are formulated as a "pre-liposome gel" referred to herein as a "gel" where a phospholipid and an aliphatic or aromatic-based acid or amine mixture capable of forming liposomes is mixed with an appropriate, concentrated aqueous solution of the hydrating compound. This gel, upon dispersion in an aqueous solution, efficiently and spontaneously forms liposomes without solvent evaporation, input of ultrasonic irradiation or any of the other means developed to insure proper formation of lipid vesicles, liposomes.

Brief Summary Text (18):

Additionally, the pre-liposome gel can be dehydrated and stored for a substantial period of time and still be capable of spontaneously forming liposomes upon rehydration.

Brief Summary Text (19):

The pre-liposome gel is extraordinarily stable, stable enough to be autoclaved for sterilization. Furthermore, water-soluble or water-insoluble substances to be encapsulated can be added to the gel and will then be incorporated into the liposomes upon dispersion of the gel. This capability has the effect of greatly enhancing the encapsulation efficiency.

Brief Summary Text (43):

Examples of liposome-forming materials include saponifiable and non-saponifiable lipids, e.g., the acyl glycerols, the phosphoglycides, sphingolipids, the glycolipids, etc. fatty acids include saturated or unsaturated alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) carboxylic acids, mono-alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.27</sub>) esters of C.<sub>sub.4</sub> .about.C.<sub>sub.10</sub> dicarboxylic acids (e.g., cholesterol hemi-succinic acid and fatty acid derivatives of amino acids in which any N-acyl carboxylic acids also are included (e.g., N-oleoyl threonine, N-linoleoyl serine, etc.). Mono- or di-alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) sulfonate esters and mono- or di-alkyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) phosphate esters can be substituted for the fatty acids. Furthermore, mono- or di-acyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) glycerol derivatives of phosphoric acids and mono- or di-acyl (C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub>) glycerol derivatives of sulfuric acids can be used in place of the fatty acids.

Brief Summary Text (44):

Additionally, the fatty acids also can be replaced by amines (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> NH.<sub>sub.2</sub>), C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> fatty acid derivatives of amines (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> CONH.<sub>sub.2</sub>), C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> fatty alcohol derivatives of amino acids (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> OOC.<sub>sub.2</sub>), and C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> fatty acid esters of amines (e.g., C.<sub>sub.8</sub> .about.C.<sub>sub.24</sub> COO.<sub>sub.2</sub>).

Brief Summary Text (46):

Although the primary components of these liposomes will be lipids, phospholipids, other fatty acids, there may also be added various other components to modify the liposomes' permeability. There may be added, for example, non-ionic lipid

components such as polyoxy alcohol compounds, polyglycerol compounds or esters of polyols; the esters of polyols and synthetic lipolipids, such as cerebrosides. Other materials, such as long chain alcohols and diols, sterols, long chain amines and their quaternary ammonium derivatives; polyoxyethylenated fatty amines, esters of long chain amino alcohols and their salts and quaternary ammonium derivatives; phosphoric esters of fatty alcohols, polypeptides and proteins.

Brief Summary Text (48):

It also has been discovered that if the lipid component itself or the substances (e.g., medicaments, biologically active compounds, cosmetics, etc.) to be encapsulated possess the aforementioned properties, the lipid composition may not require the inclusion of the fatty acids (or the amines) or the hydrating agents to form the "pre-liposome gel". For example, the mixture of dipalmitoylphosphatidylcholine (DPPC) and distearoyl phosphatidylethanolamine forms the "pre-liposome gel" or liposomes with aqueous glutamic acid solution and the mixture of DPPC and oleic acid with aqueous epinephrine solution forms the "pre-liposome gel" and liposomes.

Brief Summary Text (74):

Mixtures of liposome-forming materials, a long chain aliphatic or aromatic-based acid or amine, and one or more hydrating agents with up to 300 moles of water relative to the total solids gives a gel which forms liposomes directly therefrom upon addition of an aqueous solution. This gel can be labeled a pre-liposome gel because i.) of its structural characteristics which are essentially those of liposomes and, ii.) the gel's facility for being converted into liposomes upon dilution with an aqueous solution. Aqueous solution in excess of about 300 moles cause the beginning of liposome formation.

Brief Summary Text (79):

The pre-liposome gel, with or without the material to be encapsulated, also can be dehydrated (e.g. lyophilized) and the powder rehydrated to form liposomes spontaneously, even after a long period of storage. This capability makes the invention particularly useful for administering water-sensitive medicaments where long term pre-use storage is needed.

Detailed Description Text (12):

ii). Manufacture of Liposomes: The gel prepared in the preceding Paragraph was taken from cold storage and returned to room temperature. It was then mixed with 2 liters of phosphate buffered saline, pH 7.4. A white opaque liposome solution was formed.

Detailed Description Text (17):

Pre-Liposome Gel

Detailed Description Text (53):

To 120 mg of dipalmitoylphosphatidylcholine was added 40 mg of oleic acid to form a homogeneous paste. Forty mg of pilocarpine free base was added to 10 ml of distilled deionized water. This solution was added to the paste and heated to 45.degree. C. to form a pre-liposome gel. The resulting gel was diluted with 20 ml of phosphate buffered saline to form liposomes.

Detailed Description Text (72):

Sterile liposomes may be prepared from the heat sterilized pre-liposome gel. Alternatively, the liposome gel or the liposomes may be sterile filtered through an appropriate sterilizing filter.

Detailed Description Text (84):

A 10.0 gm aliquot of this pre-liposome gel was transferred to a 10 ml vial and lyophilized. The resulting powder formed liposomes when diluted with 5 ml of phosphate buffered saline.

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